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# Optimization of chitosan and β-cyclodextrin molecularly imprinted polymer synthesis for dye adsorption

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ABSTRACT

In this study, two types of novel molecularly imprinted polymers (MIPs) were prepared, for toxic and carcinogenic dyes adsorption. Substrates of the polymeric matrix of the two MIPs were β-cyclodextrin and chitosan. The conditions in the polymerization/imprinting stage and in the rebinding/adsorption step were optimized. The effect of a range of parameters (polymer, cross-linker, and initiator concentrations, reaction time and pH) on the selectivity and adsorption capacity of the dye-MIPs were investigated. Their dye rebinding properties were demonstrated by equilibrium batch experiments (fitting with Freundlich model) and their kinetic rates were exported by the pseudo-first order model. Additionally, a thermodynamic evaluation was carried out through the determination of enthalpy, entropy, and free energy. The selectivity of MIPs was elucidated by their different rebinding capabilities in a trichromatic mixture (composed of related structurally dyes). Regeneration/reuse of the dye-loaded polymers was evaluated via sequential adsorption–desorption cycles.



## 1. Introduction

Molecular imprinting is a method of inducing molecular recognition properties in synthetic polymers in response to the presence of template species during formation of the 3D structure of a polymer (Sellergren, 2001). The history of molecular imprinting can be traced back to the 1940s and 1950s, with studies on the affinity for dye molecules in silica gel (based on theory of Linus Pauling), which is considered to be the first imprinted material (Dickey, 1955). Since then, many research groups worldwide have studied the synthesis of highly specific molecularly imprinted polymers (MIPs) (Katz & Davis, 2000; Liu & Wulff, 2004). The main field of imprinting includes separation processes (chromatography, solid-phase extraction, and membrane separations), artificial antibodies, and sensors recognition elements (Bergmann & Peppas, 2008; Chen, Xu, & Li, 2011). MIPs present wide recognition due to their stability, ease of preparation, and low-cost potential (Liu & Wulff, 2004). The imprinting technique involves the formation of a pre-polymerization complex between the imprinted molecule (usually has relatively low molecular weight) (Liu & Wulff, 2004), and the functional polymer with specific chemical structures designed to interact with the former either covalently (Shea & Sasaki, 1989; Wulff, Grobeeinsler, Vesper, & Sarhan, 1977) or non-covalently (basic interactions) (Arshady & Mosbach, 1981; Shi, Tsal, Garrison, Ferrari, & Ratner, 1999). Based on the above consideration, the selection of a suitable polymer and optimization of appropriate polymerization conditions (cross-linkers, initiators, polymers) are crucial for the improvement of MIPs performance.

In the current study, β-cyclodextrin and chitosan reagents were selected as the substrates for MIPs synthesis, given the published data revealing the advantageous characteristics of those reagents (Asanuma, Kakazu, Shibata, Hishiya, & Komiyama, 1997; Fu, Yu, & Zhu, 2008; Guo, Xia, Hao, et al., 2005; Guo, Xia, Wang, Song, & Zhang, 2005; Yu, Deng, & Yu, 2008).

Cyclodextrins (CD), characterized as supramolecular host compounds, belong to a series of cyclic oligosaccharides. In particular, β-CD is formed by the binding of seven individual D-(+)-glucopyranose units through α-1,4-glycosidic oxygen bridges. The physical conformation of its molecular structure creates a lipophilic inner cavity with hydrophilic outer surfaces that is able to interact with a large variety of guest molecules forming non-covalent inclusion complexes (Szejtli, 1998; Yang, Long, Cao, Li, & Liu, 2008). Compared with the functional polymers of conventional MIPs, β-CD, as a new generation of functional polymer, possesses some unique advantages (Asanuma, Hishiya, & Komiyama, 2000; Yang et al., 2008). Firstly, β-CD host–guest inclusion complex and β-CD polymer orderly assembly are easily formed under mild conditions. Secondly, due to the rigidity and chirality of hydrophobic cavity, β-CD unit can form complex with the target molecule through various intermolecular interactions (van der Waals forces, electrostatic affinity, hydrophobic, dipole–dipole, and hydrogen bond interactions) during the imprinting process. This is helpful for obtaining high affinity binding sites (Asanuma et al., 1997; Piletsky, Andersson, & Nicholls, 1999; Yang et al., 2008). Moreover, owing

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to the rapid and reversible interaction between β-CD and guest, the prepared MIPs can be easily regenerated by suitable regulation and control (Hishiya, Shibata, Kakazu, Asanuma, & Komiyama, 1999). Therefore, by introducing β-CD supramolecular chemistry into molecular imprinting technique, both the preparation process of MIPs, and the recycle of the artificial receptors is more direct and simple. Furthermore, β-CD-based imprinted polymers are thought to be advantageous compared to previously employed methods of producing MIPs (Hishiya et al., 1999). The latter is due to the ease of condensation polymerization process, using simple neutral molecules such as epichlorohydrin or toluene diisocyanate (Roche, Ng, Narayanaswamy, Goddard, & Page, 2009). The polymerization process can be carried out under standard laboratory environment (25 °C, 1 atm), without the need of drastic conditions. In this case, β-CD itself can serve both as polymer to interact with template molecules, and as cross-linker to form a 3D polymer matrix (Roche et al., 2009). It is worthy to note that this system does not need any initiator or catalyst of cross-linker. The latter simplifies the whole polymerization process, avoids interfering effects caused by these molecules (such as creation of non-specific binding sites), and hinders the leaching of template during template removal process (Roche et al., 2009).

Chitosan (poly-β-(1→4)-2-amino-2-deoxy-D-glucose) is an amino-polysaccharide produced by the *N*-deacetylation of chitin, presenting abundance, non-toxicity, hydrophilicity, biocompatibility, biodegradability, and effective adsorptive ability (Rinaudo, 2006). Due to its macromolecular structure, chitosan (bearing excess of amino and hydroxyl groups) exhibits many characteristics that have recently drawn attention. The range of its applications has been enormously expanded in various fields including biotechnology, water-treatment, membranes, cosmetics, food industry, and medicine (Rinaudo, 2006). However, few works deal with the combination of chitosan in molecular imprinting (Guo, Xia, Hao, et al., 2005; Guo, Xia, Wang, et al., 2005; Fu et al., 2008; Yu et al., 2008).

Although the molecular imprinting technique has been widely used for polymer synthesis with various drugs and proteins as template molecules, the behavior of MIPs in environmental targets (e.g. industrial wastewaters) has been hardly examined (Meng, Chen, & Mulchandani, 2005; Pichon & Chapius-Hugon, 2008; Yu et al., 2008). It is notable that a limited number of works deals with the recognition of dye molecules as templates (Al-Degs, Abu-Surrah, & Ibrahim, 2009; Baggiani et al., 2009; Kyzas, Bikiaris, & Lazaridis, 2009; Yan et al., 2007). In our previous work (Kyzas et al., 2009), selectivity and rebinding properties of MIPs were studied for the recognition of dyes in dyeing mixtures (either simulated or real). However, despite the high selectivity, the adsorption/rebinding was not adequate (≤15 mg/g) (Kyzas et al., 2009). To overcome the above limitation, in the current study, other reagents were selected to increase the adsorption/rebinding sites, in line with retaining the high selectivity.

The novelty of the current study is: (i) the environmental target as template molecule used (dye), as well as the optimized conditions (ii) in polymerization/imprinting stage, and (iii) in rebinding/adsorption step employed. Consequently, variable parameters of polymerization (polymer, cross-linker, and initiator) were examined to assess the effect of "polymer cookery" in selectivity and adsorption capacity of the prepared β-cyclodextrin and chitosan based MIPs.

## 2. Materials and methods

### 2.1. Reagents

For the synthesis of MIPs of β-cyclodextrin (named hereafter as CD-MIPs), β-cyclodextrin (β-CD, ≥97%), dimethylsulfoxide (DMSO, 50 wt% in water), and toluene-2,4-diisocyanate (TDI, 95% p.a.) were supplied by Sigma–Aldrich. For the synthesis of chitosan-based MIPs (named hereafter as CHI-MIPs), high molecular weight chitosan ($3.55 \times 10^5$ g/mol), tripolyphosphate sodium (TPP, 98% p.a.), glutaraldehyde (GLA, 50 wt% in water), acrylamide (AAM, 97% p.a.), *N*,*N*′-methylenebisacrylamide (MBA, ≥99% p.a.), and potassium persulfate (KPS, ≥99% p.a.) were also purchased by Sigma–Aldrich. Remazol Red 3BS (abbreviated hereafter as RR, $C_{31}H_{19}ClN_7Na_5O_{19}S_6$, MW = 1136.31 g/mol, $\lambda_{max}$ = 588 nm, 58%, w/w) was the dye template molecule used. For the selectivity experiments, two other molecules were used as competitive/comparative dyes: (i) Remazol Blue RN (abbreviated hereafter as RB, $C_{22}H_{162}Na_2O_{11}S_3$, MW = 626.54 g/mol, $\lambda_{max}$ = 541 nm, 56%, w/w) and (ii) Remazol Yellow gelb 3RS 133% (abbreviated hereafter as RY) ($C_{28}H_{20}ClN_9Na_4O_{16}S_5$, MW = 1026.25 g/mol, $\lambda_{max}$ = 419 nm, 60%, w/w). All dyes used were supplied by DyStar (their pure dye content was taken into account for all calculations) and their chemical structures are presented in Fig. 1.

### 2.2. Preparation of CD-MIPs

Most of the imprinted polymers were prepared by the following "one-step method" (Hishiya et al., 1999). This method takes advantage of in situ formation of the β-CD-dye complex in the polymerization mixture. β-CD (5 mmol) and dye template (RR, 600 mg) were dissolved in 50 of dimethylsulfoxide (DMSO), and then 25 mmol of TDI were added. After being magnetically stirred at 65 °C for 2 h, the gel formed was chopped into pieces, washed with acetone, and ground with mortar and pestle. The polymer was sufficiently washed with hot deionized water, and hot ethanol for the removal of the residual quantities of dye templates, β-CD, and TDI. Then, the polymer was dried in vacuum at 40 °C for 24 h. As a control, blank microspheres were prepared with the same procedure in the absence of the template molecules; these polymers obtained were the non-imprinted polymers (CD-NIPs).

### 2.3. Preparation of CHI-MIPs

The preparation of CHI-MIPs was divided into two stages: (i) chitosan beads were prepared by cross-linking reactions (Kyzas, Bikiaris, & Lazaridis, 2008) and (ii) CHI-MIPs were prepared by grafting AAM on the beads formed from stage (i) (Guo, Xia, Hao, et al., 2005; Guo, Xia, Wang, et al., 2005). In particular, in the first stage, cross-linked chitosan beads were prepared by initially dissolving chitosan ($1.41 \times 10^{-6}$ mol) in 50 mL aqueous solution of acetic acid (2%, v/v). The solution was added dropwise from a pipette into an aqueous solution of GLA (0.05 mol/L), which also contained TPP ($1.36 \times 10^{-3}$ mol) at pH = 6, adjusted with an aqueous HCl solution. The formed gelled beads were stirred overnight at room temperature in the aforementioned solution. After filtration and purification by extraction with hot water in a Soxhlet apparatus for 24 h, the wet chitosan particles were stored in distilled water for further use. In the second stage, 5 g of the prepared wet cross-linked chitosan beads (use of filter to absorb the surface water), 5 mmol of AAM, 25 mmol of MBA, 20 mg of KPS, 600 mg of dye template (RR), 28 mL of sodium dihydrogen phosphate buffer (0.01 M, pH = 6.8) were put into a four-necked flask equipped with a nitrogen inlet and a mechanical stirrer. The mixture was stirred continuously under a nitrogen atmosphere and then 5 mL of $NaH_2PO_4$ buffer, containing 0.16% (w/v) of $NaHSO_3$, was added. The mixture was stirred under the nitrogen atmosphere for 2 h. To remove residuals of the polymers and template molecules, the CHI-MIPs were extracted in a Soxhlet apparatus using methanol. The extraction stage lasted for 16 h, resulting from a sum of 30–35 solvent cycles (each cycle lasted approximately 30 min). At the end of the 16-h period, the higher percentage of template molecules (~95%) was removed from the

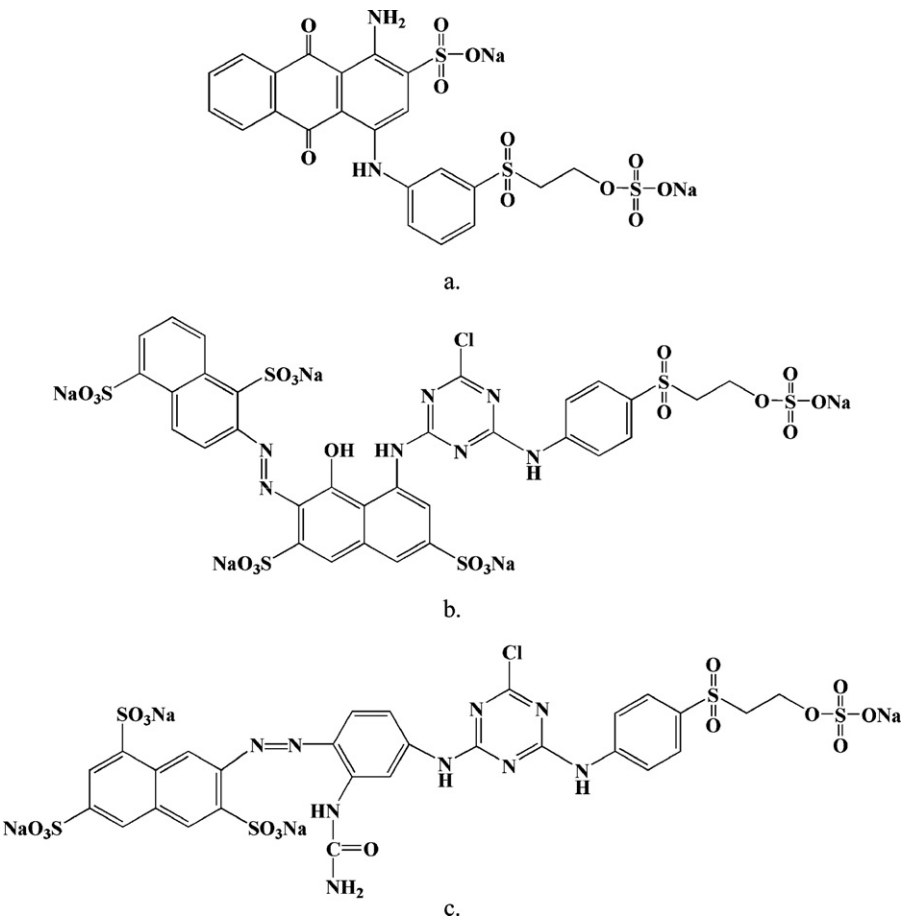


**Fig. 1.** Chemical structures of reactive dyes used: (a) Remazol Brillant Blue RN (RB); (b) Remazol Red 3BS (RR); and (c) Remazol Yellow Gelb 3RS (RY).

polymer matrix (data not shown), as measured with a UV–Vis spectrophotometer. Blank microspheres were prepared with the same procedure in the absence of the template molecules; these polymers obtained were the non-imprinted polymers (CHI-NIPs).

### 2.4. Optimization of MIPs preparation

The optimum conditions of polymer:cross-linker molar ratio, polymerization time and quantity of initiator were determined through selectivity, adsorption/rebinding, and swelling experiments carried out on MIPs prepared as previously described.

#### 2.4.1. Polymer:cross-linker molar ratio

The molar ratios of β-CD or chitosan versus the respective cross-linker (MBA or TDI, respectively) tested for each type of MIPs were 1:1, 1:2, 1:3, 1:5, 2:1, 3:1, and 5:1. Polymerization time and initiator quantity employed were 2 h and 20 mg respectively, whereas all the other conditions were as formerly described.

#### 2.4.2. Cross-linking time

Similarly, MIPs were prepared employing 1, 2, 3, 5, 12, 24 and 48 h as time of synthesis. The molar ratio of polymer versus cross-linker and the quantity of initiator used were 1:5 and 20 mg respectively, whereas all other conditions were kept as previously described.

#### 2.4.3. Quantity of initiator

The quantity of initiator tested for the optimal preparation of CHI-MIPs was 5, 10, 15, 20, 40, 60 and 80 mg. Polymer:cross-linker molar ratio and polymerization time used were 1:5 and 2 h respectively, whereas all other conditions were as formerly described.

### 2.5. Characterization

The residual concentration of dyes after adsorption experiments was calculated through the UV–Vis spectrophotometer (Hitachi U-2000). Scanning electron microscopy (SEM) observations of the MIPs were carried out using a scanning microscope (JEOL JMS-840A) equipped with an energy-dispersive X-ray micro-analytical system (Oxford ISIS 300). All the surfaces studied were coated with carbon black to avoid charging under the electron beam.

Another, important parameter of any material prepared is its swelling behavior. To study this factor, 0.5 g of sample was added in 100 mL of nitric acid and then the pH of solution was adjusted to pH = 2. Acidic conditions were selected given the optimum adsorption pH was fount to be acidic (Section 3.4.1). After immersion for 24 h at room temperature, the material was found to be completely swollen (after five measurements until there was no further weight increment). Then, the swollen MIPs were removed from the aqueous medium by filtration and the excess of solvent was removed carefully by blotting with moisturized filter paper. The samples were weighted and the percentage of swelling percentage $S$ (%) was calculated according to Eq. (1):

$$S = \left(\frac{m_t - m_0}{m_0}\right) \times 100\% \quad (1)$$

where $m_t$ (g) is the weight of the swollen sample at time $t$, and $m_0$ (g) is the initial mass of the sample before swelling ($t = 0$).

### 2.6. Adsorption/rebinding and desorption

#### 2.6.1. Effect of pH

The influence of pH on dye adsorption was studied by mixing 0.05 g of MIPs with 50 mL of an aqueous dye solution ($C_0$ = 50 mg/L). Immediately after mixing, the suspension was allowed to rebind/adsorb dyes by shaking for 24 h (contact time). The temperature was maintained constant at 25 °C using a thermostatically controlled water bath (Julabo SW-21C). The pH value, ranging between 2 and 12, was kept constant throughout the whole adsorption process with micro-additions of 0.1 M $HNO_3$ or 0.1 M NaOH. Similarly, the effect of pH on desorption of the adsorbed dye from MIPs was studied in a batch experimental set-up. After adsorption (at the optimum pH found), the samples were collected and filtered using 0.45 μm pore-sized membranes. A small fraction of the dye (~1–2%) and the adsorbent (~1%) were retained on the filter membrane; these small variations due to filtration were neglected. Desorption experiments were performed by mixing the collected amount of dye-loaded MIPs with aqueous solutions over a pH range of 2–12. After 24 h of shaking at 25 °C, the samples were collected and analyzed for determination of the optimum desorption pH. The amount of dye adsorbed or desorbed was expressed as percentage of the removal $R$ (%) (Eq. (2)), while the amount of dye uptake at equilibrium $Q_e$ (mg/g) was calculated using the mass balance equation (Eq. (3)):

$$R = \left(\frac{C_0 - C_e}{C_0}\right) \times 100\% \quad (2)$$

$$Q_e = \frac{(C_0 - C_e)V}{m} \quad (3)$$

where $C_0$, $C_e$ (mg/L) are the initial and equilibrium concentration of dye in the aqueous solution, respectively; $m$ (g) is the mass of adsorbent, and $V$ (L) the volume of adsorbate.

#### 2.6.2. Kinetics

Batch kinetic experiments were performed by mixing a fixed amount of MIPs (0.05 g) with 50 mL of an aqueous dye solution (50 mg/L). The suspensions were shaken for 24 h at pH = 2 (optimum adsorption pH value found from the pH-effect experiments) in water bath at 25 °C. Samples were collected at fixed intervals (5 min to 24 h) and analyzed using a UV–Vis spectrophotometer.

#### 2.6.3. Equilibrium/isotherms

The effect of initial dye concentration was determined by contacting 0.05 g of MIPs with 50 mL of aqueous dye solutions ($C_0$ = 10–70 mg/L). Immediately after mixing, the suspension was allowed to rebind dyes by shaking for 24 h (at pH = 2, determined as optimum value) at 25 °C. The same experiments were repeated at 45, and 65 °C. Same experiments were performed for the respective NIPs.

### 2.7. Dye analysis

The dye content of samples was measured using a UV–Vis spectrophotometer. Prior to the adsorption experiments, the effect of pH over the calibration curves and the determined maximum wavelength ($\lambda_{max}$) of each dye were studied, but no significant deviation was observed (data non shown). In the case of single-component dye solutions, the absorbance $A$ (–) was measured at its $\lambda_{max}$. In the case of mixtures, the absorbance was measured in the three wavelengths of maximum absorbencies of dyes ($\lambda_{max,RR}$, $\lambda_{max,RB}$, $\lambda_{max,RY}$) (Kyzas et al., 2009) based on the molar absorbtivity/extinction coefficients $E$ (L mol$^{-1}$ cm$^{-1}$) for the three dyes, which was calculated from the Lambert–Beer law ($A = CEZ$), $Z$ is the path length of the cell (Beer, 1852). The resulting 3 × 3 equation system (expressed by Eqs. (4)–(6)) can give the residual concentration of each dye:

$$A_{\lambda_1} = C_{RY}E_{\lambda_1,RY}Z + C_{RB}E_{\lambda_1,RB}Z + C_{RR}E_{\lambda_1,RR}Z \quad (4)$$

$$A_{\lambda_2} = C_{RY}E_{\lambda_2,RY}Z + C_{RB}E_{\lambda_2,RB}Z + C_{RR}E_{\lambda_2,RR}Z \quad (5)$$

$$A_{\lambda_3} = C_{RY}E_{\lambda_3,RY}Z + C_{RB}E_{\lambda_3,RB}Z + C_{RR}E_{\lambda_3,RR}Z \quad (6)$$

where $A_{\lambda_1}$, $A_{\lambda_2}$, and $A_{\lambda_3}$ are the absorbencies of the mixture at the $\lambda_{max}$ of RY dye ($\lambda_1$ = 419 nm), RR ($\lambda_2$ = 541 nm), and RB ($\lambda_3$ = 588 nm), respectively. $E_{\lambda_1,RY}$, $E_{\lambda_1,RB}$, and $E_{\lambda_1,RR}$ are the absorbance coefficients of pure RY, RB, RR at the $\lambda_{max}$ of RY dye. $E_{\lambda_2,RY}$, $E_{\lambda_2,RB}$, and $E_{\lambda_2,RR}$ are the absorbance coefficients of pure RY, RB, RR at the $\lambda_{max}$ of RR dye. $E_{\lambda_3,RY}$, $E_{\lambda_3,RB}$, and $E_{\lambda_3,RR}$ are the absorbance coefficients of pure RY, RB, RR at the $\lambda_{max}$ of RB dye.

### 2.8. Cycles of reuse

After adsorption experiments ($V_{ads}$ = 50 mL, $C_0$ = 50 mg/L, $T$ = 25 °C, pH = 2, $t$ = 24 h), the samples were collected and filtered, using 0.45 um pore-sized membranes. The dye-loaded MIPs were then brought into contact with 50 mL of aqueous solutions at pH = 10 (optimum desorption pH value) for the desorption of dye (other conditions as described in desorption experiments). After desorption, the MIPs were washed several times with deionized water and reused for the next cycle. Four sequential cycles of adsorption–desorption were carried out.

### 2.9. Selectivity

To verify whether the MIPs prepared are selective to their template molecules (dyes), the rebinding of those and some structurally related compounds (dyes) on the polymer were investigated. The amount of adsorption was determined with the equilibrium adsorption method (Eq. (3)). The MIPs obtained were brought into contact with a trichromatic mixture of reactive dyes ($C_0$ = 20 mg/L of each dye: RR, RB, RY). After mixing, the suspension was allowed to rebind/adsorb the dyes by shaking for 24 h (25 °C) at pH = 2 (optimum adsorption pH value).

Usually, the static distribution coefficient $K_D$ (mL/g), the separation factor $\alpha$ (–), and the relative separation factor $\beta$ (–) are utilized to evaluate the molecular selectivity of MIPs. Parameters $K_D$, $\alpha$, and $\beta$ are defined as follows:

$$K_D = \frac{Q_e}{C_e} \quad (7)$$

The selectivity of one dye versus another in the mixture is quantified by the ratio of the two partition coefficients $K_{D_1}$ and $K_{D_2}$ (for dyes 1 and 2, respectively):

$$\alpha = \frac{K_{D_2}}{K_{D_1}} \quad (8)$$

Higher value of $\alpha$ corresponds to greater selectivity; when the value of $\alpha$ is close to 1.0, the MIPs have no selectivity.

$$\beta = \frac{\alpha_M}{\alpha_N} \quad (9)$$

where $\alpha_M$ and $\alpha_N$ are the distribution coefficients of MIPs and NIPs, respectively.

The relative separation factor $\beta$ demonstrates the difference between MIPs and NIPs. Higher value of $\beta$ corresponds to greater difference. When the parameter $\beta$ is equal to 1.0, it means that there is no difference between MIPs and NIPs.

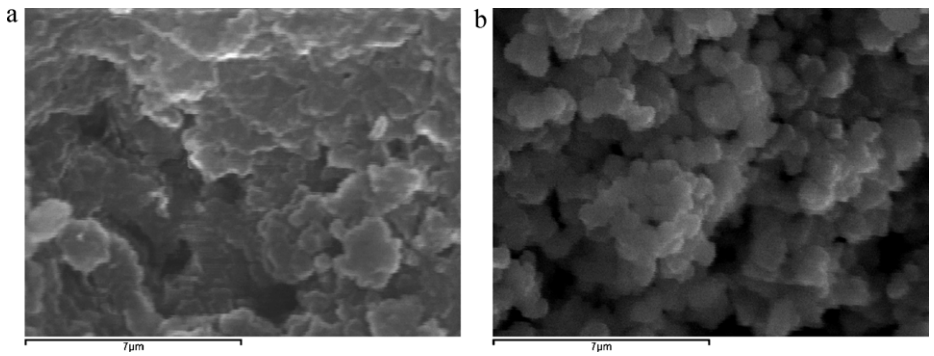


**Fig. 2.** Scanning electron micrographs (SEM) of (a) CHI-MIPs and (b) CD-MIPs.

## 3. Results and discussion

### 3.1. Characterization

Scanning electron micrographs of the prepared CHI-MIPs and CD-MIPs surfaces are presented in Fig. 2. As can be seen both MIPs exhibited a porous structure. However, larger pore size can be seen in the case of CD-MIPs, compared with CHI-MIPs. This could be attributed to the different procedures used for the preparation of MIPs. In addition, chitosan is more hydrophilic than cyclodextrins, and absorbs numerous of water molecules. After water removal a stiffer and more compact structure is formed. The majority of the particles in CD-MIPs were approximately spherical, with a lot of porous between them, which may be caused by the removal of template molecule. The MIPs like those of CD with uniform and more open structure is obviously favorable for the adsorption of the large template molecules like the used dyes. Thus, the microstructure of the prepared MIPs is expected to affect their adsorption characteristics.

### 3.2. Mechanism of dye adsorption on MIPs

#### 3.2.1. CD-MIPs

In this study, the imprinting technique selected was the non-covalent (self-assembly) type due to its well-known simplicity and the vast successful imprinting attempts reported in literature (Arshady & Mosbach, 1981; Shea & Sasaki, 1989). β-CD molecule was selected as the polymer building unit based on its capability to form inclusion complex with the dye template molecule. Dye would be accommodated partially in the hydrophobic cavities of β-CD with possible driving forces such as van der Waals and hydrophobic interactions (Liu & Guo, 2002). These interactions could be constrained to particularly one to one molecule and extended to multi-molecular interactions, which indirectly would form a uniformly distributed arrangement of β-CD and dye within the DMSO solvent (porogen). When the TDI would be introduced into the mixture, both isocyanate groups would randomly react with the hydroxyl groups on the β-CD molecules to form urethane linkages (Asanuma et al., 1997). This highly cross-linked

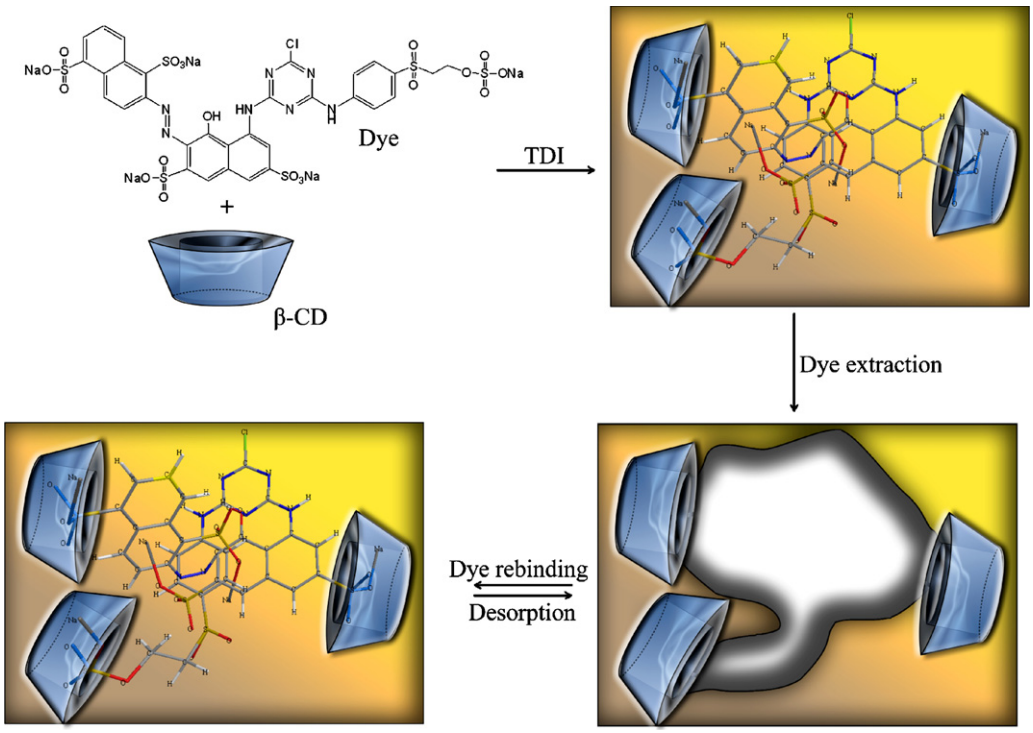


**Fig. 3.** Mechanism of CD-MIPs preparation.

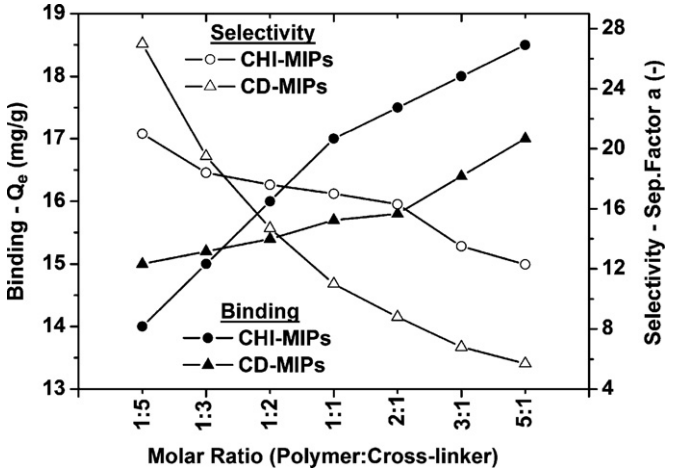


**Fig. 4.** Effect of molar ratio of polymer versus cross-linker on dye rebinding and selectivity of MIPs.

β-CD polymer matrix would then freeze all the dye molecules in a specific conformation, which, later upon removal of dye, would turn into a micro-cavity with specific shape and dye-rebinding capability. Fig. 3 illustrates the aforementioned imprinted mechanism, with the cavity created after dye extraction, and the following dye rebinding. The binding of dye molecules onto β-CD was achieved with three sulfonate groups (1 group from each side). The other sulfonate groups did not included into β-CD given the size of CD cone and the volume of dye molecule, which presented stereochemical inhibitions (Asanuma et al., 1997; Hishiya et al., 1999).

### 3.2.2. CHI-MIPs

A method quite different from the conventional MIPs synthesis was used to prepare CHI-MIPs (Yu et al., 2008). The natural polymer chitosan was adopted to prepare the MIPs through a two-step cross-linking reaction. Chitosan beads were first cross-linked slightly to prevent them dissolving in the acidic solution, and then recross-linked with excessive cross-linker to form imprinting sites after dye molecules were adsorbed on the chitosan. During the MIPs preparation, the anionic sulfonate groups of dye molecules would be adsorbed onto the protonated amino groups in chitosan molecules through electrostatic attraction. The above interaction would be stronger due to the addition of amino groups derived from AAM. Therefore, the growth of template amount is favorable for forming more effective imprinting sites during the MIPs preparation until all the available amino groups for dye are occupied.

## 3.3. Optimization of synthesis reactions for MIPs preparation

### 3.3.1. Effect of polymer:cross-linker molar ratio

Given that the two crucial reagents of imprinting are polymers or cyclic oligosaccharides in the case of CDs (both will be called as polymer for simplicity thereafter) and cross-linkers, experiments were performed to examine the optimum molar ratio of the one (polymer) versus the other (cross-linker) for the MIPs preparation. Fig. 4 depicts the effect of the aforementioned ratio on (i) dye adsorption/rebinding and (ii) selectivity of MIPs.

The impact of the latter on selectivity data (separation factor a, as described above) was similar both for CD- and CHI-MIPs. Firstly, a maximum selectivity was observed for 1:5 molar ratio (polymer:cross-linker) exporting separation factors ($\alpha$) 27.0 and 21.0 for CD-MIPs and CHI-MIPs, respectively. Increasing the percentage of polymer in the polymerization mixture, the selectivity of MIPs was shown to reduce (Fig. 4). The respective factor ($\alpha$) of 5:1 molar ratio was 5.7 and 12.3 for CD-MIPs and CHI-MIPs, respectively. However, the decrease of selectivity did not follow the same trend for the two types of MIPs. In the range of molar ratios studied, CD-MIPs lost their selectivity more sharply (from 27.0 to 5.7), while a milder decrement was observed for CHI-MIPs (from 21.0 to 12.3) (Fig. 4). This behavior was mainly attributed to the conditions of synthesis (Spivak, 2005), and the structure of MIPs prepared (Bergmann & Peppas, 2008). Furthermore, the molar ratio was shown to influence the selectivity versus the adsorption capacity. So, increasing the ratio of polymer versus cross-linker (from 1:5 to 5:1), a slight improvement in dye rebinding was observed (from 15.0 to 17.0 mg/g for CD-MIPs and from 14.0 to 18.5 mg/g for CHI-MIPs) (Fig. 4). The rebinding augmentation was slight for CD-MIPs (the difference was only 2.0 mg/g), while the respective curve of CHI-MIPs was more intense (increment of 4.5 mg/g) (Fig. 4). The excess of polymer enhanced the amount of non-specific rebinding, which lowered the overall average selectivity of MIPs. The dye templates could not react orderly/normally with the imprinted cavities of MIPs due to their rapid interaction with the excess of polymer groups. Generally, given the use of MIPs as selective adsorbents, the optimum molar ratio (polymer:cross-linker) was 1:5 for both MIPs. In this ratio, both the selectivity ($\alpha > 20$) and the rebinding properties (~15 mg/g) remained in good levels, compared to other MIPs of literature (Bergmann & Peppas, 2008; Spivak, 2005). There is a minimum amount of cross-linker necessary to form a rigid polymer network that can maintain the fidelity of the binding site. This limits the amount of functional polymer that can be used for the formation of imprinting binding sites (Spivak, 2005). Thus, optimization of polymer:cross-linker molar ratio in MIPs has to be empirically derived; however, our experimental findings are in agreement with some reports indicate that an optimum molar ratio is approximately 1:5, depending on the functional polymer used (Bergmann & Peppas, 2008; Spivak, 2005).

### 3.3.2. Effect of cross-linking time

This factor of imprinting was selected to be optimized, as it is well known to influence the adsorption and selectivity of MIPs, as well as their rigidity. Fig. 5 depicts the effect of crosslinking time on dye rebinding in line with selectivity. The selectivity was shown to follow the same trend for CD- and CHI-MIPs, as well. The lowest selectivity was observed for 1 h of crosslinking time giving separation factors ($\alpha$) 16.0 and 9.3 for CD-MIPs and CHI-MIPs, respectively. Increasing the cross-linking time, the reactions continued and the selectivity of MIPs was augmented (Fig. 5). The respective factors ($\alpha$) for CD-MIPs and CHI-MIPs found were 27.0 and 21.0 for 2 h and pick up to 27.5 and 22.6 for 48 h, respectively (Fig. 5). However, after the 2nd hour of polymerization, the selectivity was slightly improved for both cases, reaching an aggrandizement of $\alpha \sim 1$ (48 h). A sharp growth ($\alpha \sim 10$) was observed between 1st and 2nd hour. In general, MIPs formed over longer reaction periods would be more rigid and consequently should contain imprinted cavities of better defined shape, resulting in materials with higher specificity (Piletska, Guerreiro, Whitcombe, & Piletsky, 2009). The latter was in accordance with the experimental data as shown in Fig. 5.

Moreover, the cross-linking time is known to influence the adsorption/rebinding capacity. The latter was verified from data shown in Fig. 5. Increasing the time of crosslinking reactions, a slight diminishment in dye rebinding was observed (from 16.0 to 14.3 mg/g for CD-MIPs and to 13.5 mg/g for CHI-MIPs) (Fig. 5). The dye rebinding decrease of CHI-MIPs was twofold in the 2nd hour compared with CD-MIPs, and after that period, a plateau was reached. The rebinding difference between 2 and 48 h of polymerization was ~0.5 mg/g. Thus, it can be said that the optimum crosslinking time for both MIPs preparations is between 2 and 3 h, which is in agreement with previous reports (Bergmann & Peppas, 2008; Spivak, 2005).

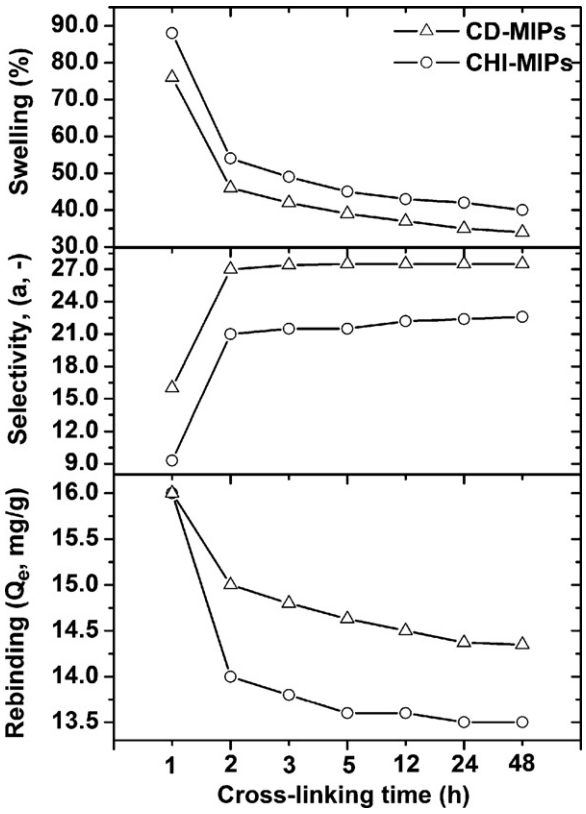


**Fig. 5.** Effect of cross-linking time on swelling, dye rebinding, and selectivity of MIPs.

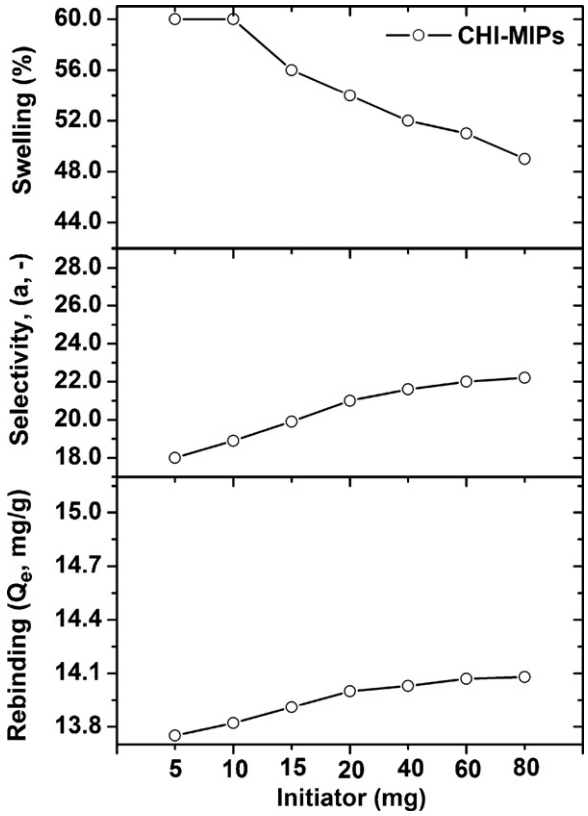


**Fig. 6.** Effect of quantity of initiator on swelling, dye rebinding and selectivity of CHI-MIPs.

To further examine the above findings, swelling experiments involving the crosslinking time and the percentage of swelling were carried out (Fig. 5). Initially, during the first crosslinking hour, the swelling percentage of MIPs was high (CD-MIPs, 76%; CHI-MIPs, 88%), while after the second hour a sharp reduction was observed (CD-MIPs, 46%; CHI-MIPs, 54%). After that time, a milder decrease occurred, reaching the final swelling percentages at 48 h (CD-MIPs, 34%; CHI-MIPs, 40%). Although the molar ratio of polymer:cross-linker was the same for both MIPs (1:5), the swelling percentage was different. The network of chitosan is known to be more flexible, allowing easily the flowing of water molecules inside (Rinaudo, 2006), in contrast to the β-cyclodextrin matrix (wide conical structure) (Hishiya et al., 1999). The swelling reduction by increasing the cross-linking time could be attributed to the formation of a more rigid and less flexible cross-linking network at higher time periods. The movement and the free space between macromolecular chains are restricted and thus the dye rebinding procedure is lowered.

### 3.3.3. *Effect of quantity of initiator*

The quantity of initiator does not seriously influence the adsorption/rebinding capacity of CHI-MIPs, as was confirmed from our findings that illustrated in Fig. 6. As can be seen the difference in capacity is ~0.3 mg/g for CHI-MIPs (5–80 mg of initiator). Still a slight improvement in selectivity was observed with aggrandizement in the quantity of initiator ($\alpha$ = 18.0 and 22.2 for 5 and 80 mg/g of initiator, respectively) (Fig. 6). In contrast, the quantity of initiator caused a more intense decrease of swelling of the CHI-MIPs prepared, which was found to be ~10% (Fig. 6, polymerization time (h)). All aforementioned data were in accordance with that mentioned in literature since the polymers formed with higher percentages of initiator would be more rigid, which should ensure that imprinting cavities with better defined shape and higher specificity would be formed (Piletska et al., 2009).

## 3.4. *Adsorption/rebinding*

After the optimization of cross-linking reactions, the next step was to evaluate the rebinding properties of MIPs prepared under different conditions (pH, contact time, temperature, and regeneration/reuse).

### 3.4.1. *Effect of pH*

One of the most important parameters controlling the dye adsorption process is pH, because it could easily influence the properties of the adsorbent, as well as the adsorbate speciation (especially in the case of dye adsorption). Fig. 7a presents the experimental results of the adsorption by CD-MIPs and CHI-MIPs under various constant pH values and equilibrium conditions. The experimental data showed that the adsorption was higher in acidic conditions than in basic ones for both types of MIPs; the higher dye adsoprtion was achieved at pH = 2 (CD-MIPs, 61%; CHI-MIPs, 52%). Increasing the pH values, the dye adsorption was shown to diminish (pH = 10: CD-MIPs, 20%; CHI-MIPs, 21%). The above pH behavior of MIPs could be attributed to the interactions between them and the adsorbate. In particular, the dye solution is full of negatively charged sulfonate groups ($SO_3^-$) after the dissociation of dye molecule. So, in acidic conditions, these groups are led to the imprinted cavities of MIPs because of the strong electrostatic forces from the protonated amino groups of chitosan-based MIPs, or possible other driving forces such as van der Waals and hydrophobic interactions from the β-cyclodextrin MIPs. The control imprinted polymers (NIPs) presented 5–7% dye adsorption for both MIPs, confirming the successful imprinted cavities formed in their matrix. This was expected since in the case of NIPs cavities with the appropriate molecule host length cannot be prepared. Other factors such as MIPs swelling or dye interactions alone with the reactive groups are not enough to cause a high selectivity for dye adsorption, as

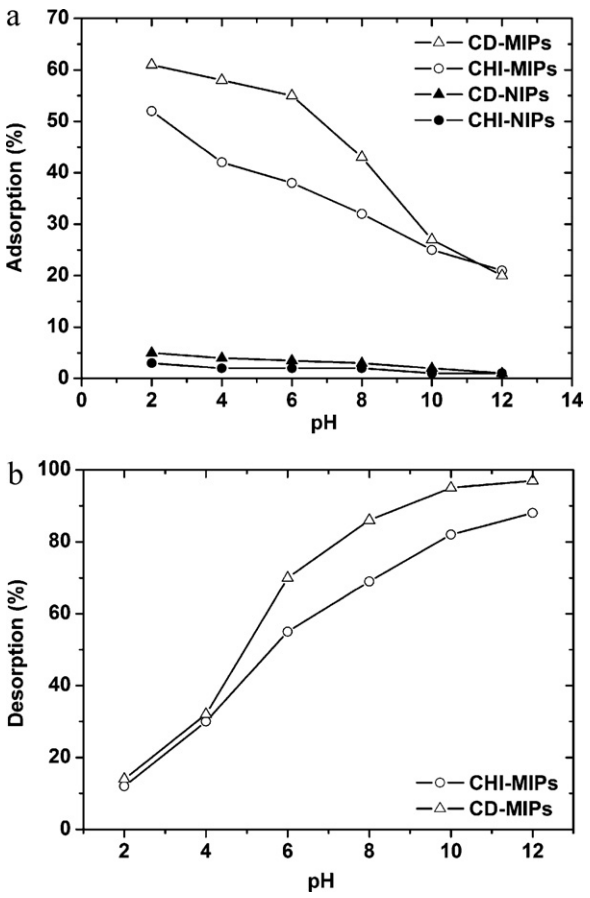


**Fig. 7.** (a) Effect of pH on adsorption/rebinding of dye template by MIPs and NIPs and (b) effect of pH on desorption of dye template by MIPs.

in the case of MIPs. Comparing the adsorption presentence in both MIPs it can be seen that this is higher in CD-MIPs. This difference could be attributed to the higher porosity of CD-MIPs compared with CHI-MIPs, which was found from SEM micrographs.

Fig. 7b illustrates the effects of pH on dye desorption from MIPs. CD-MIPs desorbed about 97% at pH = 12, while 14% at pH = 2. The enhancement of the pH of eluent led to improvement of desorption. The same trend was found for CHI-MIPs; at pH = 12 the highest desorption was achieved (88%) and at pH = 2 the lowest one (12%). So, the desorption trend was exactly the opposite of the adsorption one. This is very important in the case of MIPs reuse since adsorption can be made at low pH = 2 and desorption at high pH = 12. After desorption the prepared MIPs could be ready for new adsorption cycle.

### 3.4.2. Kinetics

After modeling attempts among pseudo-first (Eq. (10a)), -second (Eq. (10b)) and -third order equation (Eq. (10c)), the pseudo-first order equation (Eq. (10c)) was finally selected to fit the experimental kinetic data (Ho, Ng, & McKay, 2000):

$$C_t = C_0 - (C_0 - C_e)(1 - e^{-k_1 t}) \quad (10a)$$

$$C_t = C_0 - (C_0 - C_e)\left(1 - \frac{1}{1 + k_2 t}\right) \quad (10b)$$

$$C_t = C_0 - (C_0 - C_e)\left(1 - \frac{1}{(1 + 2k_3 t)^{1/2}}\right) \quad (10c)$$

where $k_1, k_2, k_3$ ($\text{min}^{-1}$) are the rate constants for the pseudo-first, -second and -third order kinetic model, respectively, and $C_0$, $C_t$, $C_e$ (mg/L) are the initial, transient and equilibrium concentrations of dye in the aqueous solution, respectively.

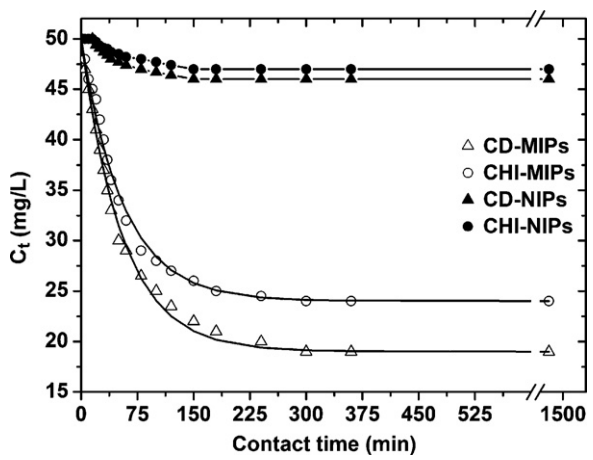


**Fig. 8.** Effect of contact time on adsorption/rebinding of dye template by MIPs (fitted to the pseudo-first order kinetic model).

The behavior of materials on the rebinding/adsorption kinetics is depicted in Fig. 8. This kinetic model was found to present the best theoretical correlation with the experimental data compared with the respective of pseudo-second and -third order models ($0.993 < R^2_{\text{ps-1st}} < 0.996$; $0.934 < R^2_{\text{ps-2st}} < 0.960$; and $0.722 < R^2_{\text{ps-3rd}} < 0.871$) (Table 1).

According to Fig. 8, a monotonous decreasing trend with high rates at the beginning of adsorption was observed for both dye-MIPs, whereas an initial steep descent was followed by a flat plateau (saturation values). Equilibrium was reached within 4 h for both MIPs. The rate constants found were $k_1 = 0.018\ \text{min}^{-1}$ for CD-MIPs and $k_1 = 0.017\ \text{min}^{-1}$ for CHI-MIPs. In general, the rate adsorption of the two types of MIPs was similar. The dye rebinding was quite fast, which could be probably due to groups of the MIPs structure with high complexation potency (Spivak, 2005).

### 3.4.3. Equilibrium/isotherms

Attempting to fit the experimental data to an affinity/isotherm model, some observations were raised. The limited application of MIPs might be due to their heterogeneity. The imprinting process typically proceeds with poor fidelity, leading to a wide distribution of association constants (Katz & Davis, 1999). The goal is to improve the heterogeneity and shift the distribution toward the higher affinity sites. Most analyses have modeled MIPs as homogeneous surfaces, grouping all the binding sites into one (Langmuir) or two (bi-Langmuir) general classes (Kermode, 1989). These homogeneous models do not readily allow comparison of changes in the distributions. The most commonly applied analysis of MIPs is the limiting slopes analysis of curved Scatchard plots, which is a form of the bi-Langmuir model (Spivak, Gilmore, & Shea, 1997). Hence, the comparison of binding properties of different or even of the same MIPs by homogeneous models is difficult.

In the current study, the model used was the Freundlich isotherm, which is believed to be the best equilibrium model for

**Table 1**
Kinetic constants of the three models used for the rebinding of RR dye onto CD-MIPs and CHI-MIPs.

| Adsorbent | Pseudo-first order | | Pseudo-second order | | Pseudo-third order | |
|---|---|---|---|---|---|---|
| | $k_1$ ($\text{min}^{-1}$) | $R^2$ (–) | $k_2$ ($\text{min}^{-1}$) | $R^2$ (–) | $k_3$ ($\text{min}^{-1}$) | $R^2$ (–) |
| CD-MIPs | 0.018 | 0.996 | 0.030 | 0.960 | 0.052 | 0.871 |
| CHI-MIPs | 0.017 | 0.993 | 0.029 | 0.934 | 0.049 | 0.722 |

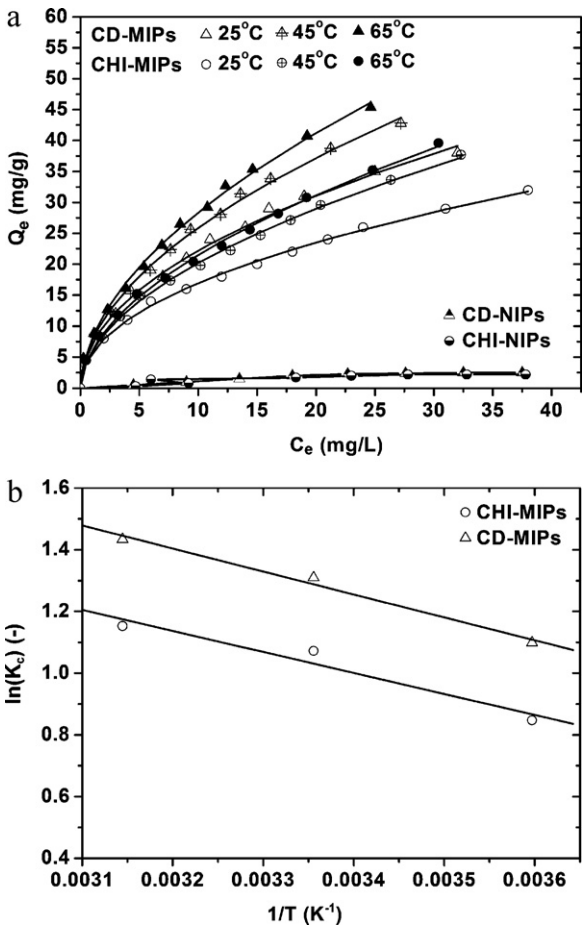


**Fig. 9.** (a) Effect of initial dye concentration on adsorption/rebinding by MIPs at 25, 45 and 65 °C (fitted to the Freundlich isotherm model) and (b) Equilibrium constant $K_c$ versus temperature ($\ln K_c$ versus $1/T$) at 20 mg/L of dye concentration (linear fitting).

MIPs (Rampey et al., 2004). This model is derived by assuming an heterogeneous surface with a non-uniform distribution of heat of adsorption over the surface and is expressed by Eq. (11) (Freundlich, 1906):

$$Q_e = K_{Fr} C_e^n \quad (11)$$

where $Q_e$ (mg/g) is the equilibrium dye concentration in the solid phase; $K_{Fr}$ ($mg^{(1-n)}\,L^n/g$) is the Freundlich constant representing the adsorption capacity; $n$ is a constant depicting the adsorption intensity/nonlinearity.

The case $n < 1$ reflects the situation, in which at higher adsorbate concentrations, it becomes more difficult to adsorb/rebind additional molecules. This could be occurred in cases, where specific binding sites become filled or remaining sites are less attractive to the adsorbate molecules. The case $n > 1$ describes a contrasting situation, in which previously adsorbed molecules lead to a modification of the surface which favors further adsorption (Schwarzenbach, Cschwend, & Imboden, 1993).

The adsorption isotherms are depicted in Fig. 9a. Experimental data were fitted to the Freundlich model and the resulting parameters are given in Table 2. The correlation coefficient ($R^2_{Fr} > 0.997$) revealed that the particular isotherm provides adequate theoretical correlation. Data showed an enhancement in the amount of dye adsorbed, when the initial dye concentration and temperature was increased. The adsorption capacity of the prepared MIPs rose up to 35 mg/g, whereas in our previous study (Kyzas et al., 2009), the maximum value reached was 12.31 mg/g (25 °C). In any temperature, the rebinding of dye was higher for CD-MIPs than CHI-MIPs.

**Table 2**
Equilibrium parameters of the Freundlich isotherm model used for the rebinding of RR dye onto CD-MIPs and CHI-MIPs (25, 45 and 65 °C).

| Adsorbent | $T$ (°C) | $K_{Fr}$ ($mg^{(1-n)}\,L^n/g$) | $n$ (–) | $R^2$ (–) |
|---|---|---|---|---|
| CD-MIPs | 25 | 7.22 | 0.49 | 0.998 |
| | 45 | 7.78 | 0.52 | 0.998 |
| | 65 | 8.20 | 0.54 | 0.999 |
| CHI-MIPs | 25 | 5.73 | 0.47 | 0.999 |
| | 45 | 6.06 | 0.48 | 0.998 |
| | 65 | 6.23 | 0.54 | 0.997 |

The Gibbs free energy change, $\Delta G^\circ$ (kJ/mol), of the adsorption process is related to the equilibrium constant, $K_c$ (–), by Van't Hoff equation (where $R$ is the universal gas constant and equal to 8.314 J/mol K) (Smith & Van Ness, 1987):

$$\Delta G^\circ = -RT \ln(K_c) \quad (12)$$

It is also related to the change in entropy, $\Delta S^\circ$ (J/mol K) and the heat of adsorption, $\Delta H^\circ$ (kJ/mol), at a constant temperature, $T$ (K), as follows:

$$\Delta G^\circ = \Delta H^\circ - T\,\Delta S^\circ \quad (13)$$

The equilibrium constant $K_c$ can be calculated as:

$$K_c = \frac{C_{Ae}}{C_e} \quad (14)$$

where $C_{Ae}$ (mg/L) is the amount adsorbed on solid at equilibrium. From the above equations, it is exported:

$$\ln(K_c) = \frac{\Delta S^\circ}{R} - \frac{\Delta H^\circ}{RT} \quad (15)$$

Thermodynamic evaluation of the dye rebinding was performed via the resulted parameters of enthalpy, entropy and free energy (as described above) at the concentration of mixture used ($C_0$ = 20 mg/L). The values of $\Delta H^\circ$ and $\Delta S^\circ$ were calculated from the slop and intercept of the plot between $\ln(K_c)$ versus $1/T$ (Fig. 9b). All thermodynamic parameters are given in Table 3. The positive values of $\Delta H^\circ$ reveal the endothermic nature of the process (Kyzas et al., 2009) and the negative values of $\Delta G^\circ$ suggest that the process is spontaneous with high preference for dye molecules (Kyzas et al., 2009). Since the adsorption is endothermic, the amount adsorbed at equilibrium is improved in line with temperature augmentation. The positive values of $\Delta S^\circ$ show the amplified randomness at the solid/liquid interface. During the adsorption, the coordinated water molecules, which are displaced by dye molecules, gain more translational entropy than is lost by dye molecules. The latter leads to higher randomness of the interaction between MIPs and dye molecules.

### 3.5. Cycles of reuse

The repeated use of MIPs, which is a key-factor for improving wastewater process economics, was estimated in four sequential

**Table 3**
Thermodynamic parameters for the rebinding of RR dye onto CD-MIPs and CHI-MIPs.

| Adsorbent | $T$ (°C) | $K_c$ (–) | $\Delta G^\circ$ (kJ/mol) | $\Delta H^\circ$ (kJ/mol) | $\Delta S^\circ$ (kJ/mol K) |
|---|---|---|---|---|---|
| CD-MIPs | 25 | 3.00 | −2.72 | 6.19 | 0.032 |
| | 45 | 3.71 | −3.24 | | |
| | 65 | 4.19 | −3.55 | | |
| CHI-MIPs | 25 | 2.33 | −2.10 | 5.66 | 0.028 |
| | 45 | 2.92 | −2.66 | | |
| | 65 | 3.17 | −2.86 | | |

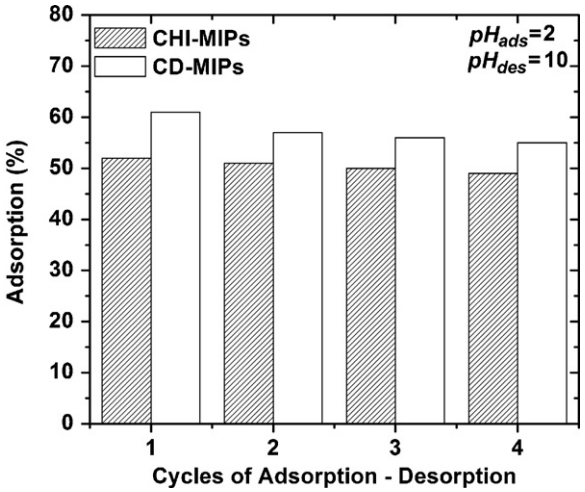


**Fig. 10.** Adsorption–desorption cycles with optimized conditions.

cycles of adsorption–desorption (Fig. 10). The loss in the dye rebinding between the first and last cycle was approximately 5 and 3% for CD-MIPs and CHI-MIPs, respectively. This gives evidence that the materials prepared can be used repeatedly without loosing significantly their adsorption capacities.

### 3.6. Selectivity

Table 4 shows the experimental results from the rebinding experiments in trichromatic dye solutions. The dye-MIPs prepared rebind with selectivity to the RR molecule (template) from a trichromatic dye solution in the presence of other reactive dyes, presenting high distribution coefficients (Table 4). The simultaneous adsorption of small amounts of the other competitive dyes (although several dyes had smaller size than the target molecules) could be attributed to the electrostatic attractions developed between MIPs and dye molecules with a non-specific manner (Piletska et al., 2009). The calculation of the relative separation factors ($\beta$) of CD-MIPs and CHI-MIPs showed that the imprinted matrix was specific and approximately 20 times greater than the non-imprinted ones (CD-NIPs and CHI-NIPs). Therefore, the selectivity of MIPs against their respective NIPs is doubtless and definite. Comparing our previous work dealing with dye-MIPs (Kyzas et al., 2009), a mild reduction in selectivity is observed, but still remains in high levels compared with those reported in literature (Bergmann & Peppas, 2008; Spivak, 2005).

**Table 4**
Effect of imprinting on selectivity of CD-MIPs and CHI-MIPs.

| Dye mixture | CD-MIPs | | CD-NIPs | | $\beta$ |
|---|---|---|---|---|---|
| | $K_D$ (L/g) | $\alpha$ | $K_D$ (L/g) | $\alpha$ | |
| RR | 3.000 | – | 0.111 | – | – |
| RY | 0.212 | 14.15 | 0.087 | 1.28 | 11.09 |
| RB | 0.176 | 17.05 | 0.099 | 1.12 | 15.20 |
| Dye mixture | CHI-MIPs | | CHI-NIPs | | $\beta$ |
| | $K_D$ (L/g) | $\alpha$ | $K_D$ (L/g) | $\alpha$ | |
| RR | 2.333 | – | 0.075 | – | – |
| RY | 0.176 | 13.26 | 0.058 | 1.29 | 10.25 |
| RB | 0.111 | 21.02 | 0.070 | 1.07 | 19.62 |

Adsorption conditions: $C_0$ = 20 mg/L for each dye; pH = 2; $T$ = 25 °C; $t$ = 24 h.

## 4. Conclusions

In this study, the substrates of the polymeric matrix of the two novel MIPs were β-cyclodextrin and chitosan. The conditions in cross-linking/imprinting stage and in rebinding/adsorption step were optimized to assess the effect of "polymer cookery" on the selectivity and adsorption capacity of the prepared dye-MIPs. The main experimental observations are summarized below:

- Increasing the percentage of polymer versus cross-linker the selectivity of MIPs was decreased, while a slight improvement in dye rebinding was observed.
- The quantity of initiator does not seriously influence neither the adsorption/rebinding capacity of CHI-MIPs nor the selectivity. In contrast, a more intense reduction of their swelling percentage was presented.
- Increasing the polymerization time, the selectivity of MIPs was improved, while a slight diminishment in dye rebinding was observed.
- Higher dye rebinding was achieved at pH = 2 and higher desorption percentages at pH = 12.
- Pseudo-first order model presented the best theoretical correlation and the equilibrium was reached within 4 h for both MIPs.
- The MIPs prepared showed capacity up to 35 mg/g. In any temperature, the rebinding of dye was higher for CD-MIPs than CHI-MIPs.
- The loss in the dye rebinding between the first cycle of regeneration and the last one was negligible, therefore the materials can be easily used repeatedly.
- The dye-MIPs prepared presented high selectivity to the RR molecule (template) from a trichromatic dye solution in the presence of other related dyes. The separation factors ($\beta$) of MIPs showed that the imprinted matrix was specific and many times greater than the non-imprinted ones.

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## References

Al-Degs, Y. S., Abu-Surrah, A. S., & Ibrahim, K. A. (2009). Preparation of highly selective solid-phase extractants for Cibacron reactive dyes using molecularly imprinted polymers. *Analytical and Bioanalytical Chemistry*, *393*, 1055–1062.
Arshady, R., & Mosbach, K. (1981). Synthesis of substrate-selective polymers by host–guest polymerization. *Macromolecular Chemistry and Physics*, *182*, 687–692.
Asanuma, H., Hishiya, T., & Komiyama, M. (2000). Tailor-made receptors by molecular imprinting. *Advanced Materials*, *12*, 1019–1030.
Asanuma, H., Kakazu, M., Shibata, M., Hishiya, T., & Komiyama, M. (1997). Molecularly imprinted polymer of β-cyclodextrin for the efficient recognition of cholesterol. *Chemical Communications*, *20*, 1971–1972.
Baggiani, C., Anfossi, L., Baravalle, P., Giovannoli, C., Giraudi, G., Barolo, C., et al. (2009). Determination of banned Sudan dyes in food samples by molecularly imprinted solid phase extraction-high performance liquid chromatography. *Journal of Separation Science*, *32*, 3292–3300.
Beer, A. (1852). Bestimmung der absorption des rothen lichts in farbigen flussigketiten. *Annalen der Physik*, *86*, 78–88.
Bergmann, N. M., & Peppas, N. A. (2008). Molecularly imprinted polymers with specific recognition for macromolecules and proteins. *Progress in Polymer Science*, *33*, 271–288.
Chen, L., Xu, S., & Li, J. (2011). Recent advances in molecular imprinting technology: Current status, challenges and highlighted applications. *Chemical Society Reviews*, *40*, 2922–2942.
Dickey, F. H. (1955). Specific adsorption. *Journal of Physical Chemistry*, *59*, 695–707.
Freundlich, H. M. F. (1906). Über die adsorption in lösungen [Adsorption in solution]. *Journal of Physical Chemistry*, *57*, 384–470.
Fu, G.-Q., Yu, H., & Zhu, J. (2008). Imprinting effect of protein-imprinted polymers composed of chitosan and polyacrylamide: A re-examination. *Biomaterials*, *29*, 2138–2142.

Guo, T.-Y., Xia, Y.-Q., Hao, G.-J., Zhang, B.-H., Fu, G.-Q., Yuan, Z., et al. (2005). Chemically modified chitosan beads as matrices for adsorptive separation of proteins by molecularly imprinted polymer. *Carbohydrate Polymers*, *62*, 214–221.

Guo, T.-Y., Xia, Y.-Q., Wang, J., Song, M. D., & Zhang, B.-H. (2005). Chitosan beads as molecularly imprinted polymer matrix for selective separation of proteins. *Biomaterials*, *26*, 5737–5745.

Hishiya, T., Shibata, M., Kakazu, M., Asanuma, H., & Komiyama, M. (1999). Molecularly imprinted cyclodextrins as selective receptors for steroids. *Macromolecules*, *32*, 2265–2269.

Ho, Y. S., Ng, J. C. Y., & McKay, G. (2000). Kinetics of pollutant sorption by biosorbents: Review. *Separation and Purification Methods*, *2*, 189–232.

Katz, A., & Davis, M. E. (1999). Investigations into the mechanisms of molecular recognition with imprinted polymers. *Macromolecules*, *32*, 4113–4121.

Katz, A., & Davis, M. E. (2000). Molecular imprinting of bulk microporous silica. *Nature*, *403*, 286–289.

Kermode, J. C. (1989). The curvilinear Scatchard plot. Experimental artifact or receptor heterogeneity. *Biochemical Pharmacology*, *38*, 2053–2060.

Kyzas, G. Z., Bikiaris, D. N., & Lazaridis, N. K. (2008). Low-swelling chitosan derivatives as biosorbents for basic dyes. *Langmuir*, *24*, 4791–4799.

Kyzas, G. Z., Bikiaris, D. N., & Lazaridis, N. K. (2009). Selective separation of basic and reactive dyes by molecularly imprinted polymers (MIPs). *Chemical Engineering Journal*, *149*, 263–272.

Liu, J.-Q., & Wulff, G. (2004). Functional mimicry of the active site of carboxypeptidase A by a molecular imprinting strategy: Cooperativity of an amidinium and a copper ion in a transition-state imprinted cavity giving rise to high catalytic activity. *Journal of American Chemical Society*, *126*, 7452–7453.

Liu, L., & Guo, X.-Q. (2002). The driving forces in the inclusion complexation of cyclodextrins. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, *42*, 1–14.

Meng, Z. H., Chen, W., & Mulchandani, A. (2005). Removal of estrogenic pollutants from contaminated water using molecularly imprinted polymers. *Environmental Science and Technology*, *39*, 8958–8962.

Pichon, V., & Chapius-Hugon, F. (2008). Role of molecularly imprinted polymers for selective determination of environmental pollutants—A review. *Analytica Chimica Acta*, *622*, 48–61.

Piletska, E. V., Guerreiro, A. R., Whitcombe, M. J., & Piletsky, S. A. (2009). Influence of the polymerization conditions on the performance of molecularly imprinted polymers. *Macromolecules*, *42*, 4921–4928.

Piletsky, S. A., Andersson, H. S., & Nicholls, I. A. (1999). Combined hydrophobic and electrostatic interaction-based recognition in molecularly imprinted polymers. *Macromolecules*, *32*, 633–636.

Rampey, A. M., Umpleby, R. J. I. I., Rushton, G. T., Iseman, J. C., Shah, R. N., & Shimizu, K. D. (2004). Characterization of the imprint effect and the influence of imprinting conditions on affinity, capacity, and heterogeneity in molecularly imprinted polymers using the Freundlich isotherm-affinity distribution analysis. *Analytical Chemistry*, *76*, 1123–1133.

Rinaudo, M. (2006). Chitin and chitosan: Properties and applications. *Progress in Polymer Science*, *31*, 603–632.

Roche, P. J. R., Ng, S. M., Narayanaswamy, R., Goddard, N., & Page, K. M. (2009). Multiple surface plasmon resonance quantification of dextromethorphan using a molecularly imprinted β-cyclodextrin polymer: A potential probe for drug–drug interactions. *Sensors and Actuators B: Chemical*, *139*, 22–29.

Schwarzenbach, R. P., Cschwend, P. M., & Imboden, D. M. (1993). *Environmental organic chemistry*. New York: John Wiley & Sons.

Sellergren, B. (2001). *Molecularly imprinted polymers, man-made mimics of antibodies and their applications in analytical chemistry, techniques and instrumentation in analytical chemistry*. Amsterdam: Elsevier.

Shea, K. J., & Sasaki, D. Y. (1989). On the control of microenvironment shape of functionalized network polymers prepared by template polymerization. *Journal of American Chemical Society*, *111*, 3442–3444.

Shi, H., Tsal, W.-B., Garrison, M. D., Ferrari, S., & Ratner, B. D. (1999). Template-imprinted nanostructured surfaces for protein recognition. *Nature*, *398*, 593–597.

Smith, J. M., & Van Ness, H. C. (1987). *Introduction to chemical engineering thermodynamics* (4th ed.). Singapore: McGraw-Hill.

Spivak, D., Gilmore, M. A., & Shea, K. J. (1997). Evaluation of binding and origins of specificity of 9-ethyladenine imprinted polymers. *Journal of American Chemical Society*, *119*, 4388–4393.

Spivak, D. A. (2005). Optimization, evaluation, and characterization of molecularly imprinted polymers. *Advanced Drug Delivery Reviews*, *57*, 1779–1794.

Szejtli, J. (1998). Introduction and general overview of cyclodextrin chemistry. *Chemical Reviews*, *98*, 1743–1753.

Wulff, G., Grobeeinsler, R., Vesper, W., & Sarhan, A. (1977). Enzyme analogue built polymers: 5. Specificity distribution of chiral cavities prepared in synthetic-polymers. *Macromolecular Chemistry and Physics*, *178*, 2817–2825.

Yan, S., Gao, Z., Fang, Y., Cheng, Y., Zhou, H., & Wang, H. (2007). Characterization and quality assessment of binding properties of malachite green molecularly imprinted polymers prepared by precipitation polymerization in acetonitrile. *Dyes and Pigments*, *74*, 572–577.

Yang, Y., Long, Y., Cao, Q., Li, K., & Liu, F. (2008). Molecularly imprinted polymer using β-cyclodextrin as functional monomer for the efficient recognition of bilirubin. *Analytica Chimica Acta*, *606*, 92–97.

Yu, Q., Deng, S., & Yu, G. (2008). Selective removal of perfluorooctane sulfonate from aqueous solution using chitosan-based molecularly imprinted polymer adsorbents. *Water Research*, *42*, 3089–3097.